PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
LBP27PC00		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 03/00454	17.01.2003	17.01.2002
International Patent Classification (IPC) or both national classification and IPC		
C12N5/10, C12N5/10		
Applicant		
LONZA BIOLOGICS PLC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06.06.2003	24.03.2004
Name and mailing address of the international preliminary examining authority:	Authorized Officer
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/00454

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-28 as originally filed

Claims, Numbers

1-9 as originally filed

Drawings, Sheets

1/7-7/7 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-9
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. This application concerns a glutamine-auxotrophic human cell for production of a protein of interest which grows in glutamine-free medium. To achieve glutamine independency, the cell was transfected with glutamine synthetase (GS). Furthermore, the cell was adapted to grow in suspension and in serum-free medium. Also claimed is the use of GS as a selectable marker in glutamine auxotrophic human cells.

2. Reference is made to the following documents:

   D1: WO 99/05267 A (BRANDT MICHAEL ;FRANZE REINHARD (DE); PESSARA ULRICH (DE); BOEHRIN) 4 February 1999 (1999-02-04)
   D2: BEBBINGTON C R ET AL: "HIGH-LEVEL EXPRESSION OF A RECOMBINANT ANTIBODY FROM MYELOMA CELLS USING A GLUTAMINE SYNTHETASE GENE AS AN AMPLIFIABLE SELECTABLE MARKER" BIO/TECHNOLOGY, NATURE PUBLISHING CO. NEW YORK, US, vol. 10, no. 2, 1992, pages 169-175, ISSN: 0733-222X
   D3: ZHOU WEICHANG ET AL: "Large scale production of recombinant mouse and rat growth hormone by fed-batch GS-NSO cell cultures." CYTOTECHNOLOGY, vol. 22, no. 1-3, 1996, pages 239-250, ISSN: 0920-9069
   D4: WO 97/08307 A (KIM KEE WON ;KIM SUN YOUNG (KR); KIM TAE HAN (KR); LEE SUN YOUNG () 6 March 1997 (1997-03-06)

3. Claims 1-9 appear to be novel over the cited prior art.

4. Claims 1-9 lack inventive step according to Art. 33 (3) PCT as the subject-matter is obvious over D2 in combination with D1.

4.1 The closest prior art document is D2, which is directed to the same purpose as the subject-matter claimed in the present invention, namely high-yield expression of a

foreign DNA and growth in glutamine-free medium.

4.2 D2 shows high-level expression of a recombinant antibody in mouse myeloma cells. The myeloma cells express GS and were adapted to suspension culture in serum-free, glutamine-free medium (Fig. 5; p. 172, right-hand column, paragraphs 3-4). GS and the recombinant cB72.3γ4 antibody were present in separate DNA constructs when introduced into the cells (Fig. 3).
It appears thus that the only difference between D2 and the subject-matter of the present claims is that human cells (HT1080 fibrosarcoma) are used instead of murine (myeloma) cells.
The advantage of using human cells is the distinct sialylation pattern. However, this feature is well known to the person of skill in the art and it would be obvious to use human cells for this reason when a protein is produced for therapy in humans.

4.3 D1 discloses the use of human HT1080 cells for high-level production of EPO. Gene-amplification was achieved in D2 by the DHFR/MTX system which is an alternative to the GS/MSX system of the present invention. Thus, it is known that HT1080 cells are well suitable for high-level production of EPO and the expression can be enhanced by selectable markers.
It appears thus that the skilled person would have considered the teachings of both D2 and D1 to arrive at the solution proposed in the present invention.
Hence, the subject-matter of claims 1-9 is not inventive over D2 in combination with D1.

4.4 D3 discloses mouse myeloma cells expressing GS and rat growth hormone in suspension culture in serum-free, glutamine-free medium.
D4 discloses avian cells transfected with a vector expressing EPO and containing GS as a selectable marker and for gene amplification.
Hence, it appears that D3 and D4 are affecting inventive step in a manner analogous to D2.